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13. ABSTRACT *(Maximum 200 words)*

The ability to selectively manipulate and probe molecules at the cellular and sub-cellular level is critical both in basic research and in the development of biotechnology applications. One powerful method to do this is to use small particles the interact with individual cells or specific molecules, and which respond to an electric or magnetic field. This approach has found widespread application in cell sorting, biosensing, and studies of mechanical properties of cells using magnetic particles. However, a significant limitation of these magnetic carriers is that they have only a single chemical functionality per particle. In this research program, we are beginning development of a new type of magnetic carrier: multifunctional magnetic nanowires. These nanowires will be able to carry out multiple tasks e.g. binding multiple types of molecules, probling chemical activity in specific regions of a cell, and responding to light as well as to magnetic fields. This was a one-year "Seedling Project" whose goal was to carry out key initial experiments to demonstrate the feasibility of creating and employing multifunctional magnetic nanowires for biotechnology and defense-relevant applications. The results obtained have provided a knowledge base from which to start development of a wide range of uses of the nanowires. This work is currently ongoing under DARPA/AFOSR support. The specific research objectives of this project included: (i) Functionalization of signe- and multi-component nanowires, (ii) Demonstration of binding interactions between nanowires and cells, and (iii) Magnetic manipulation of cells and nanowires. We have made significant progress in all three areas.

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Final

~~Annual~~ Performance Report

Demonstration of Surface Modification and Cell Interactions of Asymmetric Magnetic Nanowires

AFOSR Agreement Number F49620-01-1-0384

September 1, 2002

Reporting Period: September 1, 2001 – May 14, 2002

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1. Statement of Objectives.

The ability to selectively manipulate and probe molecules at the cellular and sub-cellular level is critical both in basic research and in the development of biotechnology applications. One powerful method to do this is to use small particles that interact with individual cells or specific molecules, and which respond to an electric or magnetic field. This approach has found widespread application in cell sorting, biosensing, and studies of mechanical properties of cells using magnetic particles. However, a significant limitation of these magnetic carriers is that they have only a single chemical functionality per particle. In this research program, we are beginning development of a new type of magnetic carrier: multifunctional magnetic nanowires. These nanowires will be able to carry out multiple tasks e.g. binding multiple types of molecules, probing chemical activity in specific regions of a cell, and responding to light as well as to magnetic fields.

This was a one-year "Seedling Project" whose goal was to carry out key initial experiments to demonstrate the feasibility of creating and employing multifunctional magnetic nanowires for biotechnology and defense-relevant applications. The results obtained have provided a knowledge base from which to start development of a wide range of uses of the nanowires. This work is currently ongoing under DARPA/AFOSR support.

The specific research objectives of this project included: (i) Functionalization of single- and multi-component nanowires, (ii) Demonstration of binding interactions between nanowires and cells, and (iii) Magnetic manipulation of cells and nanowires. We have made significant progress in all three areas, as described below.

2. Status of effort.

Experiments were carried out to address the three goals of this project: surface functionalization of single and multi-component nanowires, demonstration of nanowire-cell interactions, and the development of the capability to manipulate the nanowires magnetically in suspensions. Selective functionalization of two-part Au:Ni nanowires was demonstrated. High purity and yields were achieved in magnetic separations using Ni nanowires. Magnetic assembly and manipulation of single cells with nanowires was shown to be feasible.

3. Accomplishments/New Findings.

Introduction

This was a one-year "Seedling Project" whose goals, as stated above, were to explore the feasibility of creating and employing multifunctional magnetic nanowires for biotechnology and defense-relevant applications. Here we describe some of the results obtained.

1. Chemical Functionalization of Nanowires

We have made important initial advances in the functionalization of magnetic nanowires with molecular compounds, including surface functionalization of one- and two-part nanowires. This key advance is an important first step toward the application of multifunctional nanowires to biotechnology. Below we highlight this work with some detail of the experimental approach. Fluorescence microscopy is compatible with the low concentration and relatively small surface area of the nanowires and has therefore been used to quantify the surface chemistry

Surface Functionalization of Nanowires. Previous research on planar surfaces has shown that molecular functional groups can react specifically and selectively with metal or metal oxide surfaces. Some relevant examples include the binding of carboxylic acids to metal oxide surfaces and thiols to gold surfaces. Here we have exploited these findings and probed a simple question: Will known interfacial chemistry at planar surfaces translate to nanowire surfaces? The answer appears to be yes and in all systems investigated a strong correlation between the chemistry of planar surfaces and that of nanowire surfaces exists.

Figure 1 shows an idealized schematic of how [8,13-bis(1-hydroxyethyl)-3,7,12,17-tetramethyl-21 *H*,23 *H*-porphine-2,18-dipropionic acid], abbreviated HemIX, might bind to the oxide surface of a nickel magnetic nanowire. HemIX has two carboxylic acid groups coupled to the porphyrin ring through ethylene bridges. There is sufficient flexibility in the ethylene spacer that the two carboxylic acid groups can bind simultaneously to the same wire, presumably as the carboxylate type binding shown.

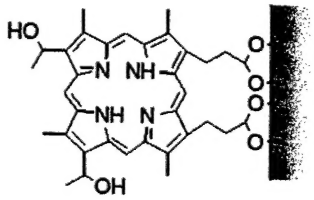

Figure 1. Schematic of binding of HemIX to a Ni nanowire surface.

Nickel nanowires do in fact react with

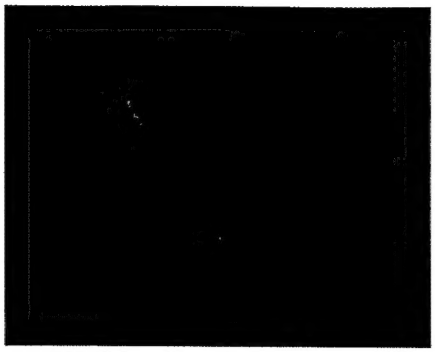

Figure 2. Fluoresence micrograph of a Ni nanowire functionalized with HemIX.

HemIX. A 12-micron long nanowire reacted with HemIX in ethanol yields the fluorescent wire shown in Figure 2. Control experiments performed with a porphyrin that does not contain carboxylic acid groups (tetraphenyl porphyrin) and bare nickel nanowires under otherwise identical conditions reveal no fluorescence. Concentration dependent binding data was well described by the Langmuir adsorption isotherm model from which the adduct formation constant $K = 9 \times 10^6\ M^{-1}$ was abstracted. The large adduct formation constant reflects the strong metal oxide-carboxylic acid interaction consistent with previous reports on planar surfaces and ideal for biotechnology applications.

Reacting gold nanowires with HemIX under identical conditions leads to negligible surface binding as determined from fluoresence measurements. Two-part nanowires, comprised of a nickel and a gold segment, were also investgated. To help prevent the binding of HemIX to the gold segment, the two-part wires were also reacted with a long chain thiol, nonylmercaptan, which is known to bind strongly to gold. The idealized structure that was envisioned is shown schematically in Figure 3.

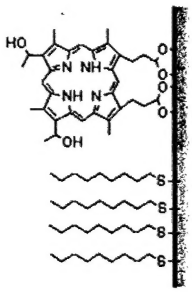

Figure 3. Schematic of binding of HemIX and an alkanethiol to a two-segment Ni (gray) – Au (yellow) nanowire.

Figure 4. Left: visible light image of 2-segment Ni/Au nanowire, with the nickel section of the nanowire in the upper right and the gold in the lower left. The gold section was colored yellow to be made more clear. Right: fluoresence image of the same nanowire. One sees that the nickel segment has a fluorescent monolayer of HemIX while the gold segment does not.

Strong evidence for this selective functionalization of two-part nanowires was obtained from visible and fluoresence microscopy data like that shown in Figure 4. One sees that the nickel segment has a fluorescent monolayer of HemIX while the gold segment does not. Selective functionalziation has been achieved by either a one-step process where the wires were exposed to HemIX and nonylmercaptan simultaneously or by a sequential functionalization where the wires were first exposed to the nonylmercaptan and then the HemIX. Both procedures yielded selective functionalization of the nanowires like that shown above. Publications describing this work are in preparation.

2. Cell-Nanowire Interactions.

One of the most important basic applications of magnetic particles in biology is magnetic separation. In this process magnetic particles are bound to one or more components of a heterogeneous mixture of cells or biomolecules. These moieties may then be collected with a magnet and removed from the mixture. This process is typically carried out using superparamagnetic beads. As an important first step in developing techniques for cell manipulation with nanowires, we have demonstrated that magnetic separation can be done with nanowires. Preliminary experiments have shown high purities and yields in separations using NIH 3T3 mouse fibroblast cells, and that the nanowires show potential to be competitive with magnetic beads. Detailed quantitative experiments will be carried out over the next few months.

3. Magnetic Manipulation of Nanowires and Cells

3.1 Magnetic assembly of cells. We have previously developed a method of self-assembly of nanowires from suspension. This works by allowing the nanowires to settle on flat substrates where they interact through dipolar forces and tend to aggregate. If the nanowires are initially randomly oriented in the fluid, then this process yields random collections of nanowires. Controlled assembly may be achieved by applying a small external field $H < 10$ G. By prealigning the suspended nanowires, this field suppresses the tendency toward random aggregation, and leads to the formation of extended head-to–tail nanowire chains. These chains can become quite long, ultimately extending over hundreds of microns. We have demonstrated that this chaining process can be used to assemble cells. Beginning with suspensions of cells bound to Ni nanowires obtained from the separation process described above, we can assemble chains of cells, as shown in Figure 5. Although the cell-surface adhesion forces are

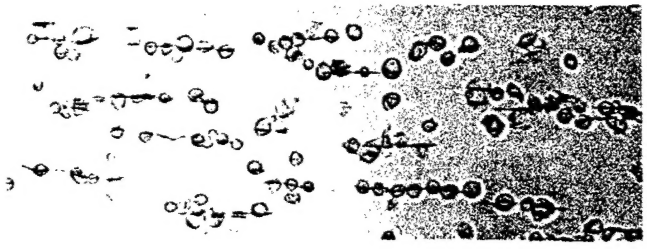

Figure 5. Chains of NIH-3T3 cells produced by magnetic self assembly of individual cells bound to magnetic nanowires.

sufficient to separate the nanowires as the cells attach to the substrate, this method appears to show considerable promise for controlling the initiation of cell culture, and potentially ultimately for in-vitro tissue engineering.

3.2 Magnetic trapping of cells We have recently developed another way to control and position magnetic nanowires from suspension, which we term magnetic trapping. As shown schematically in Figure 6, this works by attracting nanowires with strong local magnetic fields generated by lithographically patterned micro-magnets. If the gap between the micromagnets is appropriately chosen, then single nanowires can be trapped and made to bridge the gap, as shown in Figure 7. In a non-biological context, we are working to exploit this technique to make electrical measurements on single nanowires. For the purposes of this project, we have shown that this technique can be used to trap and localized single cells that have first been bound to a nanowire, as shown in Figure 8. This has potential applications in a variety of contexts, from localized control of the initiation of cell culture and studies of single cells, to biosensing and interfacing to microelectronics

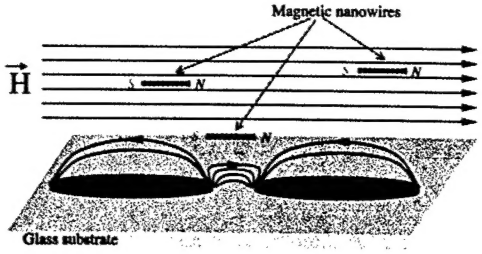

Figure 6. Schematic of magnetic trapping process wherein nanowires are captured by local fields of patterned micromagnets.

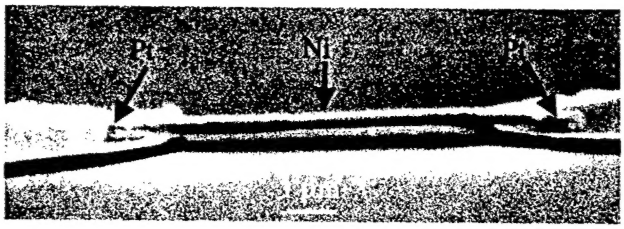

Figure 7. Scanning electron micrograph of a magnetically trapped Pt/Ni/Pt nanowire

Figure 8. A NIH-3T3 cell that was first bound to magnetic nanowire, and then magnetically trapped by lithographically patterned micromagnets (dark elipses).

5. Personnel Supported:

Faculty:

Christopher S. Chen,	Department of Biomedical Engineering
Chia-Ling Chien,	Department of Physics and Astronomy
Gerald. J. Meyer	Department of Chemistry
Daniel H. Reich	Department of Physics and Astronomy
Peter C. Searson	Department of Materials Science and Engineering

All participating faculty had one-half calander month of their salary cost-shared by JHU to support their efforts on this project

Graduate Students:	**Portion of Total Salary from AFOSR funds**
Laura Bauer	100%
Nira Birnbaum	100%
Min Chen	100%
Anne Hultgren	100%
Monica Tanase	100%

6. Publications:

1. M. Tanase, D. M. Silevitch, A. Hultgren, L. A. Bauer, P. C. Searson, G. J. Meyer, and D. H. Reich, "Magnetic Trapping and Self-Assembly of Multicomponent Nanowires," J. Appl. Phys. **91**, 8549 (2002).
2. C. L. Chien, L. Sun, M. Tanase, L. A. Bauer, A. Hultgren, D. M Silevitch, G. J. Meyer, P. C. Searson, and D. H. Reich, "Electrodeposited Magnetic Nanowires: Arrays, Field-Induced Assembly, and Surface Functionalization," J. Magn. Magn Mater. 249, 146 (2002).

7. Interactions/Transitions:

a. Participation/presentations at meetings, conferences, seminars, etc.:

1. A. Hultgren, M. Tanase, C. S. Chen, G. J. Meyer, and D. H. Reich, "Cell manipulation using magnetic nanowires," Contributed presentation, American Physical Society March Meeting, Indianapolis, IN, March, 2002.
2. G. J. Meyer, "Surface functionalization of multicomponent nanowires," Seminar, William Patterson University, April, 2002.
3. G. J. Meyer, "Surface functionalization of multicomponent nanowires," Seminar, City University of New York, April 2002.

b. Consultative and advisory functions to other laboratories and agencies: None

c. Transitions:

We have begun a collaboration with Polysciences, Inc., of Warrington, PA. Polysciences is a leading manufacturer of small magnetic particles for biological applications such as cell separation. Interactions, including exchange of materials and technical discussions, are currently underway.

8. New discoveries, inventions, or patent disclosures.

1. Surface functionalization of one- and two-component magnetic nanowires.
2. Magnetic assembly of cells.
3. Magnetic trapping of single cells using magnetic nanowires.
4. D. H. Reich, C. S. Chen, C. L. Chien, G. J. Meyer, and P. C. Searson, "Multifunctional Magnetic Nanowires," U. S. Patent Application filed No. 10/143,813, May 15, 2002.

9. Honors/Awards: None.